Ckk1p microtubule localization regulates microtubule sliding but not spindle midzone integrity, a role attributed to Ase1p in fission yeast. A single microtubule-binding site within the motor domain suggests that like MKlp1, protein association with the stalk is needed to mediate other microtubule interactions. Premature overexpression of Ckk1 can stabilize bipolar spindles in a compromised Kinesin-5 *cut7-22ts* strain, overriding the opposing effect of Kinesin-14 Pkl1. However in wild type cells overexpressed Ckk1p results in spindle collapse. Upon removal of *pkl1,* the ability of Ckk1p overexpression to cause spindle collapse is reduced, however pre-prophase spindle elongation occurs. The result is non-central, off-side anaphase A segregation of chromosomes. Ckk1p represents a novel chromatin-binding Klp in fission yeast, absent in budding yeast, that has multiple roles in stabilizing spindle assembly, equatorial chromosome alignment and spindle elongation and does not fall cleanly into either Kinesin-6 or Kinesin-10 families of Klps.

2623-Pos Board B593

Binding Dimeric Kinesin-like proteins to Tubulin: Analysis of Microtubule and Pole Determinants

Jessica Allen, Erika Sadeghi, Janet L. Paluh.
Rensselaer Polytechnic Institute, Troy, NY, USA.

The interaction of kinesin-like proteins (Klps) with microtubules or α/β-tubulin heterodimers is fundamental to the mitotic mechanism through regulation of microtubule organization and dynamics. Since the discovery of conventional Kinesin in 1985, and kinesin-like proteins (Klps) beginning in 1990, information on the molecular nature of this interaction has been vague. In part its analysis has awaited elegant crystallographic data that now allows us to visualize, albeit still at low resolution, the site of interaction of monomeric and dimeric Klps on microtubules. In addition the availability of sequenced genomes provides bioinformatic profiling of conserved tubulin elements. This lab is investigating determinants of dimeric Klp/tubulin interactions. We recently defined for the first time, since the discovery of kinesin over 20 years ago, the binding site on tubulin for a Klp. That tubulin is the microtubule organizing center (MTOC) protein γ-tubulin and the Klp, dimeric Kinesin-14 Pkl1. Kinesin-14 Klps are ubiquitous in eukaryotes and oppose spindle bipolarity mediated by the Kinesin-5 family. The Kinesin-14/γ-tubulin interaction is effective as a prominent mechanism to oppose spindle bipolarity. We demonstrated that its disruption, by mutating the γ-tubulin site for Kinesin-14 binding, restored spindle bipolarity and viability in a normally impaired Kinesin-5 *cut7-22^{ts}* strain. We are examining additional molecular features of the Kinesin-14/γ-tubulin binding site and determining its relationship to Klp/β-tubulin interactions. By site-directed mutagenesis we have altered similar residues in β-tubulin helix 11 and are generating chimeric β- and γ-tubulins with switched helix 11 domains. Analysis of the tubulin derivatives in vivo and in vitro will allow us to determine affects on Klp/tubulin interactions of both plus- and minus-end directed Klps and on microtubule dynamics and organization.

2624-Pos Board B594

Structure-activity Relationships In Synthetic Systems Of Coupled Motor Proteins

Jonathan W. Driver, Kenneth D. Jamison, Pamela E. Constantinou, Arthur R. Rogers, Michael R. Diehl.
Rice University, Houston, TX, USA.

Motor proteins play an essential role in regulating the internal organization of the cytoplasm by maneuvering a wide variety of vesicles and organelles. Many of these transport processes depend on the ability of motors to function collectively, in groups that contain more than one motor molecule. Utilizing protein engineering and DNA-self-assembly techniques, we have created experimental models of these multi-motor systems. Our synthetic assemblies afford precise control over the number of coupled motors in a construct, as well as their relative positions and the mechanical compliance of the linkage between each motor and the solid support to which it is anchored. Furthermore, the molecular architecture of these assemblies has been characterized using both bulk methods and single-molecule microscopy techniques. Our initial optical trapping experiments revealed rich behavior in a system of two coupled kinesin motors; the force-velocity relationship for individual constructs reveals that coupled kinesin motors can move at higher-than-predicted velocities when under high loads. Additionally, the system's stepping dynamics appears to vary significantly with applied load. Here, we discuss our efforts to characterize the effect of mechanical compliance and inter-motor separation upon these behaviors. Understanding these structure-activity relationships is critical to our broader goal of a comprehensive, mechanistic model of collective motor protein transport. Such a model would provide important insight into the process by which a cell controls its internal order.

2625-Pos Board B595

Visualizing Collective Dynamics of Nonprocessive Motors in Membrane Tube Formation

Paige M. Shaklee[1,2], Line Bourel-Bonnet[3], Marileen Dogterom[2], Thomas Schmidt[1].
[1]Leiden University, Leiden, Netherlands, [2]FOM Institute AMOLF, Amsterdam, Netherlands, [3]Universite Louis Pasteur Strasbourg, Strasbourg, France.

The emergent collective behavior of motor proteins plays an important role in intracellular transport. For example, processive motors (kinesins) work in concert to extract membrane tubes from membrane compartments. In this case, it has been shown that motor proteins form dynamic clusters that can collectively generate enough force to extract membrane tubes (Koster *et al*, *PNAS* 2003, Leduc *et al*, *PNAS* 2004). Recent *in vitro* experiments have shown that nonprocessive motors (ncds) can also extract membrane tubes: here, tubes show distinct phases of persistent growth, retraction, and an intermediate regime characterized by dynamic switching between the two (Shaklee *et al*, *PNAS* 2008). The physical mechanism by which nonprocessive motors collectively mediate membrane tube formation has, however, not yet been experimentally investigated.

We use a minimal *in vitro* model system where motors are specifically attached to a fluorescently labeled lipid on Giant Unilamellar Vesicles (GUVs) to examine motor behavior during membrane tube formation. Motors collectively extract membrane tubes from the GUV as they walk on underlying microtubules. FCS and FRAP experiments reveal a directed flow as processive motors walk at typical speeds (<500nm/s) along the underlying microtubule and accumulate at the tip of the growing membrane tube. However, fluorescence correlations in time show that nonprocessive motors exhibit purely diffusive behavior, decorating the entire length of the microtubule lattice with diffusion constants at least 10 times smaller than that of a lipid-motor complex freely diffusing in a lipid bilayer (1um^2/s); FRAP experiments confirm this longer timescale for exchange of motors in the tube. These results suggest that membrane-bound motor proteins interacting with a microtubule are restricted in their diffusive motion, potentially due to fast local binding/unbinding to the microtubule lattice. This restriction likely promotes dynamic motor accumulation needed for membrane tube regulation.

2626-Pos Board B596

Experimental Realization of a Feedback Controlled Flashing Ratchet

Benjamin J. Lopez, Nathan Kuwada, Heiner Linke.
Physics Department University of Oregon, Eugene, OR, USA.

A flashing ratchet transports diffusive particles by on and off switching of an asymmetric periodic, ratchet shaped spatial potential. Recent theory work has predicted that the use of a feedback algorithm based on particle positions can increase velocity by up to an order of magnitude compared to periodic flashing. Experimental implementation of feedback control is also predicted to show current reversals and synchronization effects. Feedback control could be used to model and understand the gating mechanisms in linear, dimeric molecular motors that lead to processivity.

We have successfully implemented feedback control of a flashing ratchet system and observed the predicted increase in velocity. We compare two different feedback algorithms for small particle numbers. The maximum instantaneous velocity method (MIV) considers the force on all the particles when the ratchet is on, and the maximum net displacement (MND) method considers the distance of the particles compared to a certain reference point. We also find that through manipulation of this reference point, the algorithm can be further improved to be more tolerant of feedback delay times. We find good agreement with Langevin simulations that take into account the feedback delay time and spatial sampling of the potential by the finite-sized microspheres.

We use an optical line trap to realize a flashing ratchet, and through real-time image analysis we achieve fast feedback with implementation delay time of 5 ms. We use an acousto-optic deflector to create the line trap by scanning an optical tweezer fast enough that a trapped silica microsphere feels a time-averaged potential.